Norio SAHASHI*: **Morphological and taxonomical studies on Ophioglossales in Japan and the adjacent regions (10)** Spore morphology and cytogeographical distribution on *Japanobotrychium lanuginosum***

佐橋紀男*: 日本および近隣産ハナヤスリ目の形態学的分類学的研究 (10) アリサンハナワラビの胞子形態と細胞地理学的分布

According to Jermy & Walker (1977), *Japanobotrychium lanuginosum* (*Botrychium lanuginosum*) ranges from central Himalaya across China to Taiwan, south wards to Sri Lanka (Ceylon) through Indian Subcontinent and also to Luzon, Sumatra, Java and New Guinea. In central Nepal, the author found this species at various habitats, i.e., amid grasses on open hill slopes, on humus soil in the forest floor, very often epiphytic on mossy tree trunks, and sometimes petrophytic on rocks.

The most recent cytological study on this species in New Guinea has been made by Jermy & Walker (1977). They recorded n=180, and 2n=270 chromosomes and hybrid meiosis. The basic number for the Botrychiaceae is well established as 45, and the New Guinean specimens were found to be octoploid (8X) and hexaploid (6X). On the other hand, n=90 (4X) chromosomes were reported by Ninan (1958) from S. India, Verma & Loyal (1960) from N. India, and Manton & Sledge (1954) from Sri Lanka. However, all these counts (n=90) were got merely from Indian Subcontinent. The author found three spore types in the present study among the specimens from both of Indian Subcontinent and New Guinea. These spore types suggest that three cytotypes exist in this species. To clarify the cytogeographical distributions of the three cytotypes, the author used fifty materials shown in Tab. 1.

Materials and methods

Spores were collected mainly from herbarium specimens and prepared for the light microscope and SEM following Sahashi (1976). For cytological study, a small portion of the frond bearing young sori was fixed in Newcomer's fluid

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Tab. 1. Locality and ploidy.

Specimen No.	Locality	Ploidy
1	Nepal, Bilbatay Bhanjang, 2200 m alt., 6305419 (TI)	6X
2	ibid., 6305423 (TI)	4X
3	Nepal, Sinduwa, 1100 m alt., 6305422 (TI)	4X
4	ibid. 2100 m, 6305420 (TI)	4X
5	Nepal, Kakani, 2200 m alt., 6305452 (TI)	4X
6	Nepal, Bhuspate Danra, 2900 m alt., 6305431 (TI)	4X
7	Nepal, Helambu, 2200 m alt., 79324 (TOHO)	4X
8	ibid. 2400 m, 78019 (TOHO)	4X
9	ibid. cult. at Funabashi, 460398 (TNS)	4X*
10	Nepal, Wallich 48 (BM)**	4X
11	India, Dargeeling, 2400 m alt., 725749 (TI)	4X
12	India, Naini Tal, 2000 m alt., 165445 (TAI)	4X
13	India, Kashmir, 1500 m alt., 939949 (BO)	4X
14	India, Tehri, Gamble 27249 (P)	4X
15	India, Khasi Hills, 1400 m alt., Clark 16132 (K)	6X
16	Bhutan, Rhoguna-Ritang, 2200 m alt., Nakao 329 (KYO)	4X
17	Thailand, Chiang Mai, 1100–1800 m alt., 9981 (TI)	4X
18	ibid. 1330–1900 m, 2676 (TI)	4X
19	ibid. ca. 500 m, 9829 (TI)	4X
20	ibid. 900–1800 m, 15737 (KYO)	4X
21	North Vietnam, Chapa, Hayata (TI)	4X
22	Sri Lanka, Freeman 190823 (BM)	8X
23	China, Yunnan, Ducloux 3377 (P)	8X
24	ibid. Delavay 40 (P)	8X
25	ibid. Forrest 7228 (K)	4X
26	Taiwan, 169613 (TAI)	4X
27	Taiwan, Alishan, 78020 (TOHO)	4X
28	ibid. Hsen 15775 (TUA)	4X
29	ibid. Iwasaki 15781 (TUA)	4X
30	Taiwan, Kaohsiung, Okamoto (KYO)	4X
31	Philippines, Luzon, Vanoverbergh 2796 (P)	4X
32	Indonesia, Isl. Alor, 1500 m alt., 93818132 (BO)	6X
33	Indonesia, Isl. Sulawesi, 1200 m alt., 939945 (BO)	8X
34	ibid. 1000 m, Smith (BM)	8X
35	ibid. Yoshida 998 (KYO)	8X
36	Indonesia, Isl. Java, 2000 m alt., 939939 (BO)	8X
37	Indonesia, Isl. Lombok, 1500 m alt., 939941 (BO)	8X
38	Indonesia, Isl. Bali, 2000 m alt., 939944 (BO)	8X
39	Papua New Guinea, Western Highlands, 2600 m alt., (BO)	6X
40	Papua New Guinea, Milne Bay, 2200 m alt., Cvultwell (K)	6X
41	Papua New Guinea, Kokoda, 2000 m alt., Croft et al. (K)	4X
42	Papua New Guinea, Madang, 1680 m alt., T8351 (BM)	6X
43	ibid., T8333 (BM)	8X***
44	ibid., T8332 (BM)	6X***
45	ibid., T8336 (BM)	6X***
46	ibid., T8360 (BM)	4X
47	ibid., T8352 (BM)	8X
48	ibid 1500 m, A3889 (BM)	6X
49	ibid., A3886 (BM)	4X
50	ibid., A3885 (BM)	4X

* Specimen from which chromosome number was counted in this study (Fig. 1).
** Type specimen of *Botrychium lanuginosum*, deposited in BM.
*** Chromosome number counted by Jermy & Walker (1977).

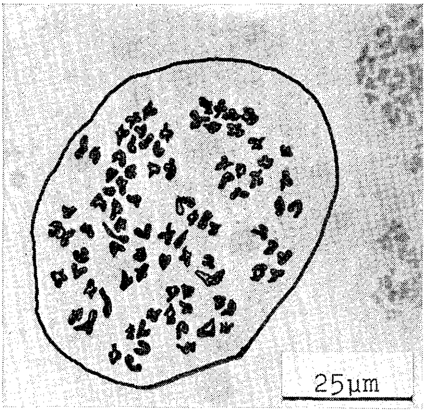

Fig. 1. Chromosomes of *Japanobotrychium lanuginosum* from Nepal, showing ca. 90 chromosomes in meiosis (voucher specimen no. 460398 in TNS).

followed by the usual aceto-carmine squash method.

Observations

Spore morphology: The spores of tetraploid specimen (specimen No. 9 in Tab. 1) which has ca. 90 chromosomes in meiosis (Fig. 1) were used for the typical spore descriptions of the tetraploid type. Among the spore characters, the author observed particularly equatorial diameter, exine thickness, laesura margin and ultrastructure of exine surface. Three spore types recognized by the author are as follows:

1) Tetraploid type (Figs. 2. A–C, 3. A–D)

Spores normal, small, subtriangular to triquetrous in polar view. Equatorial diameter ranges 25–37 μm, an average of 30 μm. Exine is ca. 2 μm thick with sculptures. Laesura margin (margo) is ca. 3.5 μm broad (if present). Reticulum-like pattern on the distal face is usually small, and finely striated or fingerprint-like pattern is clearly observed (Fig. 3. D). The spores of the type specimen in BM (specimen No. 10 in Tab. 1) were too young (Fig. 3. A). But the surface of the grain is allied to the typical tetraploid (Fig. 3. B), though it is fairly shrunken.

2) Hexaploid type (Figs. 2. D–F, 3. E, F)

Spores abnormal, almost of all are sterile, often large alete (without laesurae) spores are recognized (Fig. 3. E). The alete spores ranges 38–65 μm, an average of 50 μm diameter. Exine is variable in thickness, and the surface is irregularly foveo-fossulate pattern (Fig. 3. E). More or less finely striated sculptures are observed, but are not so clear (Fig. 3. F).

3) Octoploid type (Figs. 2. G–I, 3. G–H)

Spores normal, more or less large, subtriangular to triquetrous in polar view. Equatorial diameter ranges 34–44 μm, an average of 39 μm. Exine is ca. 3.5 μm thick with sculptures. Laesura margin is ca. 5 μm broad (if present). Reticulum-like pattern on the distal face is usually large (Fig. 3. G), and finely

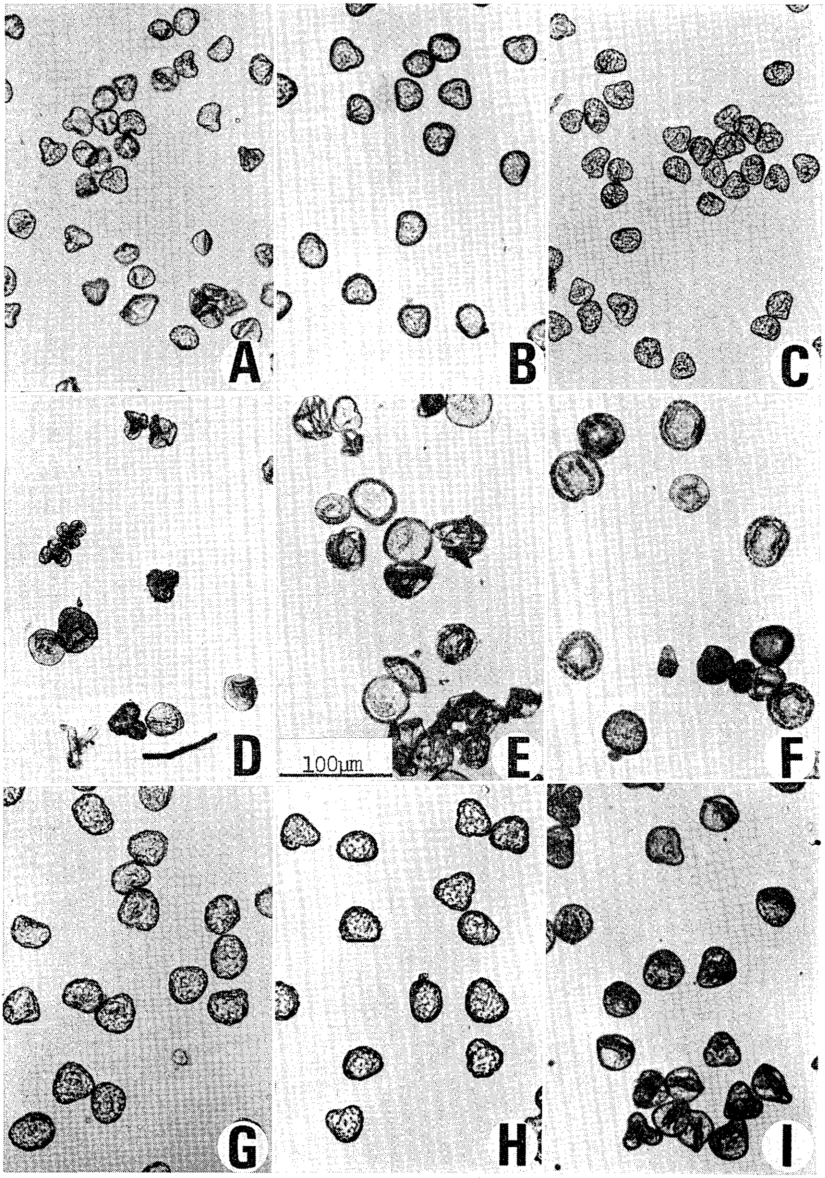

Fig. 2. Spores of three cytotypes. A–C: Tetraploid. A. Specimen No. 9. B. No. 50. C. No. 31. D–F: Hexaploid. D. No. 32. E. No. 48. F. No. 1. G–I. Octoploid. G. No. 23. H. No. 47. I. No. 38.

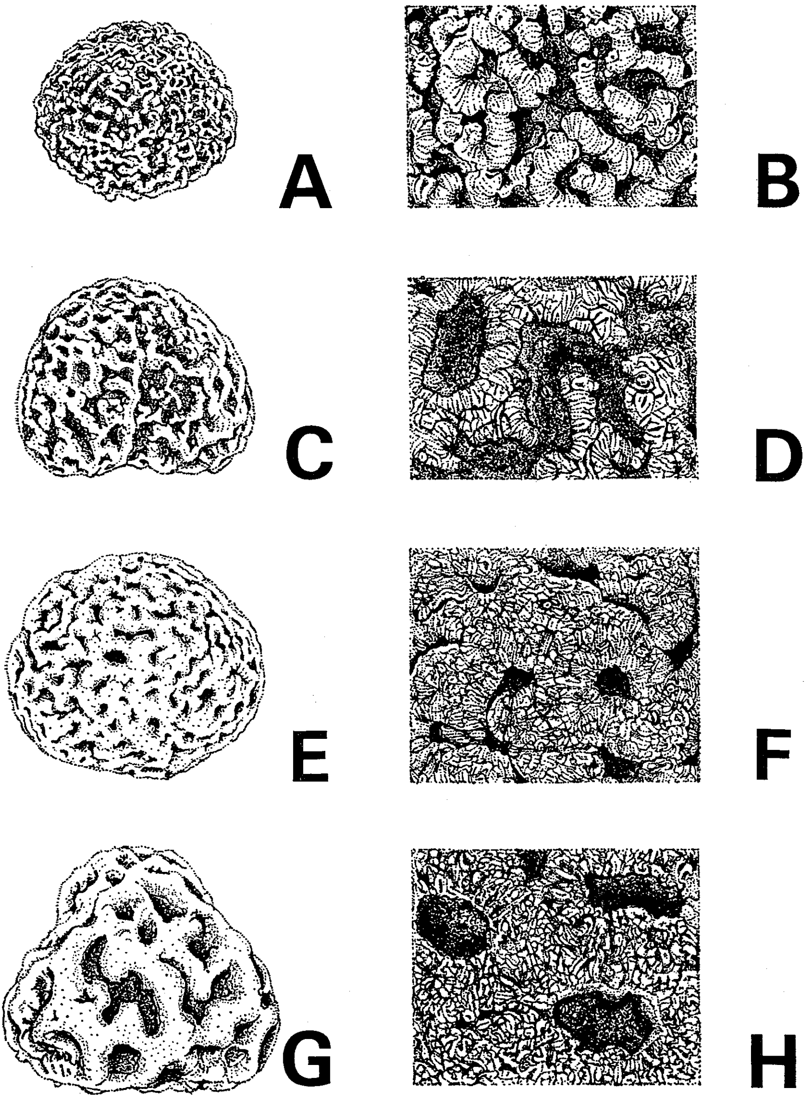

Fig. 3. Spores of three cytotypes. A–D: Tetraploid type. A, B. From Nepal, type specimen in BM, showing an immature grain, verruca-like projections faintly striated. C, D. From Papua New Guinea, A3885 in BM, showing reticulum-like sculptures finely striated on the irregular ridges. E, F: Hexaploid type, Nepal, 6305419 in TI, showing an alete spore more or less finely striated on the irregular ridges. G, H: Octoploid type, from China, Dulcoux 3377 in P, showing more or less large reticulum-like sculptures, and with finely striate or fingerprint-like pattern on the irregular ridges.

striated or fingerprint-like pattern is clearly observed (Fig. 3. H).

In the present study the author could not obtain any spore samples from the hexaploid or octoploid specimens. Instead, he observed stomata-size of not only tetraploid specimen from Nepal but also hexaploid and octoploid specimens from New Guinea. The results of the correlation between the equatorial diameter of the spore and the length of the stomata are shown in Fig. 4. It is found that mean spore-size of the octoploid type (39 μm) is distinctly larger than that of the tetraploid type (30 μm). Actually, the spore-sizes of the specimen, which shows the stomata-size as large as the octoploid specimen ($n=180$) with unknown number of chromosomes, are apparently larger than the spores of tetraploid specimen ($n=$ca. 90).

Chromosome numbers: Some plants of this species were transplanted to Funabashi, Chiba Pref. from central Nepal in 1979. Three specimens from them were observed at meiosis, and ca. 90 bivalents were counted as shown in Fig. 1. No irregular meiosis was observed.

Size of stomata: The length of stomata was measured in 20 samples per specimen (Fig. 4, left). The mean size is 58 μm in the tetraploid (specimen No. 9 in Tab. 1), 76 μm in the hexaploid (Nos. 44, 45) and 84 μm in the octoploid (No. 43) respectively. It seems that the cytological differences are well reflected in the size of stomata. However, the stomata-sizes of specimens presumed to be tetraploid or hexaploid according to the morphological study of spore are not always in the same ranges as shown in the tetraploid ($n=$ca. 90) or hexaploid ($2n=270$) specimens (Fig. 4).

Cytogeographical distribution: Fig. 5 shows the cytogeographical distribution of the three cytotypes mainly based on the spore morphology. A particular trend of distribution is not found in any cytotypes so far as the present study is concerned. However, the tetraploid has not been found at any islands in Indonesia, and the octoploid is absent in the Himalayan region. Unexpectedly the hexaploid is rarely found in both regions.

Discussion

Some palynological researches on this species were carried out by Nakamura & Shibasaki (1959), Academia Sinica (1976) and Kato & Sahashi (1977). However, both of palynological and cytogeographical observations are made at first in the present study.

It is well known that in the intraspecific polyploidy of ferns the higher

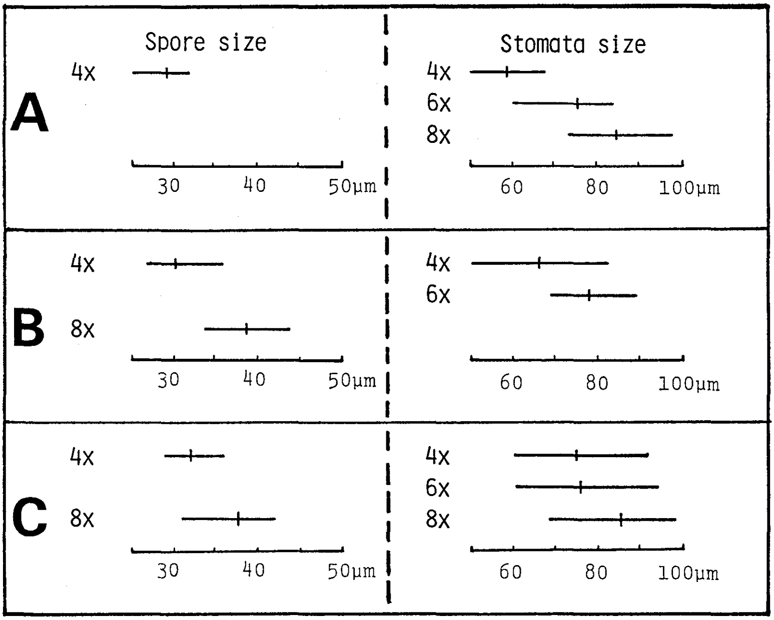

Fig. 4. Ranges and means of sizes of spore and stomata. A: Specimen Nos. 9 and 43-45 in Tab. 1. B: Specimens from Nepal, Bhutan, Thailand, North Vietnam, Sri Lanka, China and Taiwan; Nos. 1-8 and 10-30. C: Specimens from Philippines, Indonesia and Papua New Guinea; Nos. 31-42 and 46-50.

grade of ploidy is accompanied by a relative increase in sizes of spores and stomata. In the present study, the size of spores were shown apparently correlated with ploidy levels (Fig. 4. A-C, left), but the cytological differences were not always reflected in the size of stomata (Fig. 4. C, right). Nakato & Mitui (1979) already reported just the same results as the above mentioned using *Diplazium subsinuatum*.

Jermy & Walker (1977) found two cytotypes, i.e., octoploid (8X) and hexaploid (6X) based on their collections of *Japanobotrychium lanuginosum* from New Guinea in 1964. They also suggested that the tetraploid (4X) may also be present in New Guinea. Fortunately, the author observed the spore types which might have been derived from the different cytotypes, i.e., tetraploid type (Figs. 2. B, 3. C, D), hexaploid type (Fig. 2. E) and octoploid type (Fig. 2. H). Furthermore, the tetraploid type shows almost the same spore morphology

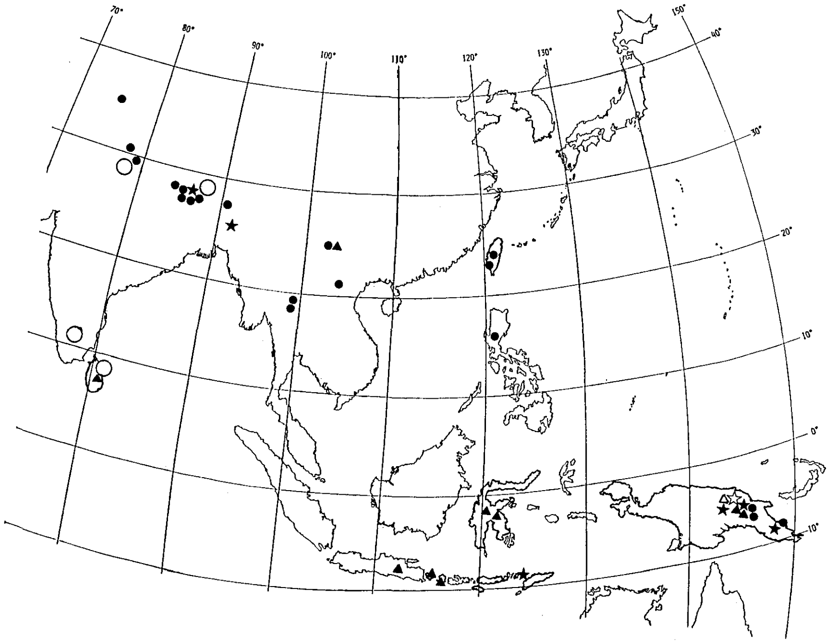

Fig. 5. Distributional map of three cytotypes. ● tetraploid. ★ hexaploid. ▲ octoploid (based on spore morphology). ○ tetraploid. ☆ hexaploid. △ octoploid (based on chromosome numbers).

as those of the typical tetraploid in Nepal. In fact it is noted that the specimen generated by hybridization generally has abnormal and sterile spores. Therefore, from the palynological views of the present study, a proof of the existence of tetraploid (4X) in New Guinea is showed.

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References

Academia Sinica 1976. Sporae Pteridophytorum Sinicorum, Peking. Jermy, A.C. & T.G. Walker 1977. Gard. Bull. **30**: 293-298. Kato, M. & N. Saha-

shi 1977. Acta Phytotax. Geobot. **28**: 143-151. Manton, I. & W. A. Sledge 1954. Phil. Trans. Roy. Soc. London **238**B: 127-185. Nakamura, J. & T. Shibasaki 1959. Research reports of the Kochi Univ. **8**: 1-11. Pl. I-VIII. Nakato, N. & K. Mitui 1979. Journ. Jap. Bot. **54**: 129-136. Pl. III-VI. Ninan, C. A. 1958. Cytologia **23**: 291-316. Sahashi, N. 1976. Journ. Jap. Bot. **51**: 326-339. Pl. VII-XI. Verma, S. C. & D. S. Loyal 1960. Curr. Sci. **29**: 69-70.

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アリサンハナワラビは広く東南アジアに分布し，ハナワラビ科のなかでは形態学的に他の種類とは異なった興味ある幾つかの点を持っている。この種の細胞学的な研究は断片的になされているが，広い分布域でのまとまった研究はない。そこでこれまでの細胞学的な知識と胞子の形態から，この種の分布域内においてこれまで知られている3種類の種内倍数体(4X，6X，8X)がどのように分布しているかを，胞子の形態を重視して，50個体の倍数性を調べた。6Xと8Xは染色体のカウントされた個体からの胞子は得られなかったが，種内倍数化にともなう胞子の形態の変化を把握し，あわせて気孔の大きさも参考とし，無理なく3種類の種内倍数体の分布図を作製した。その結果，倍数化による明確な分布域の境界はみいだせなかったが，インド亜大陸にはおもに4Xが分布し，インドネシアの島々には8Xが広く分布しているようである。また6Xもどちらの分布域にもわずかにみいだせる。Jermy らがニューギニアに4Xの存在を示唆したが，今回の胞子の観察結果から十分その存在を認めることができる。

□上野益三：**博物学史論集** 595+32pp. 1984. 八坂書房，東京. ¥15,000. 過去20年にわたる論文52篇の転載をふくむ著作集。「本草学と博物学」の章には小野蘭山，畔田翠山，飯沼慾斎，岩崎常正らの人物と業績を記し，「植物」では薬園や腊葉帖にふれ，「西洋博物学断章」ではビュルゲル，フッカー父子などの記事，「人物」では明治以後の矢田部良吉，他7人のナチュラリストを記述，「動物」では象，カワウソ，ニホンオオカミなどにふれ，「動物学」では動物学史に関する5篇を入れ，「本」では本とのふれあいを述べる。巻頭に10図版，巻末に著者の博物学史関係著作目録あり。本書の索引，著者経歴抄を付す。日本博物学史と，その第1人者の著者を知る上に欠かせない書物である。

(木村陽二郎)